PATENT Docket No.: 1038-844

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Loosmore, et al.
Application No.:		09/210,995
Filing Date	:	December 15, 1998
Title	:	Multi-Component Vaccine Comprising At Least Two Antigens From Haemophilus influenzae To Protect Against Disease
Grp./AU	:	1645
Examiner	:	Jana A. Hines

October 16, 2003

APPEAL BRIEF AND REQUEST FOR EXTENSION OF TIME
TOTAL PAGES 78

BY FACSIMILE **703-872-9307**

The Commissioner of Patents and Trademarks
BOX AF,
Washington, D.C.
20231, U.S.A.

Dear Sir:

Introduction

This Appeal Brief is submitted pursuant to applicant's appeal from a Final Rejection of claims 6-24 dated October 22, 2002. A Notice of Appeal was filed on April 22, 2003. The enclosed Credit Card Payment Form includes the prescribed fee. In the event of underpayment or overpayment please apply any additional charges or refunds to USPTO Deposit Account Number 500715. Three copies of the Appeal Brief are provided herewith.

Extension of Time

Petition is hereby made under the provisions of 37 CFR 1.136(a) for an extension of four months of the period for filing this Appeal Brief. The enclosed Credit Card Payment Form includes the prescribed fee. In the event of underpayment or overpayment please apply any additional charges or refunds to USPTO Deposit Account Number 500715.

(1) **Real Party of Interest**

The real party of interest with respect to this patent application is Aventis Pasteur Limited. Assignments from the inventors to Aventis Pasteur Limited are recorded at Reel 010239/0462 on December 15, 1998.

(2) **Related Appeals and Interferences**

The appellants, the appellants' legal representatives and assignee, are unaware of any pending appeals or interferences which will directly affect or be affected by or have a bearing on the Board's decision in the pending appeal.

(3) **Status of Claims**

This application was filed with claims 1-24. In the response dated September 10, 1999 to the Office Action of April 13, 1999 claims 1 and 22 were amended.

Claims 1-24 were finally rejected in an Office Action dated October 14, 1999. Claims 1-5 were cancelled in a reply brief dated October 19, 2001. Claims 6 to 24 are pending and the subject of this appeal and appear in Appendix I hereto.

(4) **Status of Amendments**

This application was filed with claims 1-24. Claims 6-24 are pending and no amendments were filed subsequent to this final rejection.

(5) **Summary of Invention**

The present invention is directed to an immunogenic composition for conferring protection in a host against disease caused by *Haemophilus influenzae,* including otitis media. The composition comprises at least two different antigens of *Haemophilus influenzae,* a high molecular weight (HMW) protein of a non-typeable strain of *Haemophilus influenzae,* particularly an HMW 1 or HMW 2 protein of the non-typeable strain (page 5, lines 19 to 23), and an analog of *Haemophilus influenzae* Hin47 protein having a protease activity which is less than about 10% of that of the natural Hin47 protein (page 5, line 24 to 31). The invention is further directed to compositions were HMW protein is present in an amount which enhances the immune response in the host to the Hin47 protein and while the individual immunogenicities of the proteins in the composition is not impaired. The Hin47 protein having a protease activity

which is less than about 10% of that of the natural Hin47 protein can have specific amino acid mutations to achieve this, claims 9-14. The HMW 1 or HMW 2 protein can be produced recombinantly (claim 16) or from specific strains (claim 17). The composition of the present invention can further comprise an adjuvant (claim 18 and 19) and the components can be in specific quantities of about 25 to about 100 μg of the Hin47 protein analog, and about 25 to about 100 μg of the HMW protein (claim 20). The invention is also directed towards methods of immunizing a host against disease caused by infection with *H. influenzae* (claim 24).

(6) Issues

The sole issue for consideration is the rejection of claims 6 to 24 under 35 USC 103(a) as being unpatentable over Barenkamp et al in view of Loosmore et al.

(7) Grouping of Claims

All claims do not stand or fall together, but rather each claim is individually patentable.

(8) Argument

(a) Background to the Invention

Haemophilus influenzae is the cause of several serious human diseases, such as meningitis, epiglottitis, septicemia and otitis media. There are six serotypes of *H. influenzae*, designated a to f, that are identified by their capsular polysaccharide. *H. influenzae* type b (Hib) was a major cause of bacterial meningitis until the introduction of several Hib conjugate vaccines in the 1980's. Vaccines based upon *H. influenzae* type b capsular polysaccharide conjugated to diphtheria toxoid, tetanus toxoid, or *Neisseria meningitidis* outer membrane protein have been effective in reducing *H. influenzae* type b-induced meningitis. The other serotypes of *H. influenzae* are associated with invasive disease at low frequencies, although there appears to be an increase in the incidence of disease caused by these strains as the incidence of Hib disease declines. Non-encapsulated or non-typeable *H. influenzae* (NTHi) are also responsible for a wide range of human diseases including otitis media, epiglottitis, pneumonia and tracheobronchitis. The incidence of NTHi induced disease has not been affected by the introduction of the Hib vaccines.

Otitis media is the most common illness of early childhood, with 60 to 70% of all children, of less than 2 years of age, experiencing between one and three ear infections. Chronic

otitis media is responsible for hearing, speech and cognitive impairments in children. *H. influenzae* infections account for about 30% of the cases of acute otitis media and about 60% of chronic otitis media. In the United States alone, treatment of otitis media costs between 1 and 2 billion dollars per year for antibiotics and surgical procedures, such as tonsillectomies, adenoidectomies and insertion of tympanostomy tubes. It is estimated that an additional $30 billion is spent per annum on adjunct therapies, such as speech therapy and special education classes. Furthermore, many of the causative organisms of otitis media are becoming resistant to antibiotic treatment. An effective prophylactic vaccine against otitis media is thus desirable.

(b) The Present Invention

Having regard to the above Background, it would be desirable to provide efficacious combination vaccines comprising *H. influenzae* components containing selected relative amounts of selected antigens. The present invention provides an immunogenic composition for conferring protection in a host against disease caused by infection with *H. influenzae,* including otitis media.

The immunogenic composition comprises at least two different antigens of *H. influenzae,* one of which is a high molecular weight (HMW) protein of a non-typeable strain of *Haemophilus influenzae* and the other of which is an analog of *Haemophilus influenzae* Hin47 protein having a protease activity which is less than about 10% of that of the natural Hin47 protein as claimed in claim 6, and all claims dependant thereon.

Claim 7 recites that the HMW protein is present in an amount which enhances the immune response in the host to the Hin47 protein. Claim 8 recites that the HMW protein is present in the recited amount while the individual immunogenicities of the proteins in the composition is not impaired. The applicants data supports such results.

(c) Rejection of claims 6-24 under 35 USC 103(a).

Claims 6 to 24 have been finally rejected under 35 USC 103(a) as being unpatentable over Barenkamp (WO 87/36914) in view of Loosmore. Barenkamp teaches high molecular weight proteins of non-typeable *H. influenzae* identified as HMW1, HMW2, HMW3 and HMW4, which are characterized by molecular weight and sequence information. Loosmore et al teach an analog of *H. influenzae* Hin47 protein with reduced protease activity. It is

submitted that these references lack any motivation to combine two different antigens of *H. influenzae,* namely a non-proteolytic Hin47 protein of Loosmore et al with the HMW proteins of Barenkamp et al in an immunogenic composition. Claim 6 defines an immunogenic composition for conferring protection in a host against disease caused by *Haemophilus influenzae,* which comprising two components, namely:

an analog of *Haemophilus influenzae* Hin47 protein having a decreased protease activity which is less than 10% of that of natural Hin47 protein, and a high molecular weight (HMW) protein of a strain of non-typeable *Haemophilus influenzae.* Thus, particularly in quantities where the immune response of the Hin47 protein is enhanced by the HMW protein (claim 7) and in which the individual immunogenicities of the proteins is not impaired (claim 8).

The Barenkamp et al reference describes the HMW protein while the Loosmore et al reference describes the non-proteolytic Hin47 analog. It is the applicants position that neither reference provides the motivation to combine the two immunogens in a single composition as required by claim 6, and by dependency all claims on appeal.

As the Examiner points out, both references contain the statement:

> "The immunogenic composition of the invention may further comprise at least one other immunogenic or immunostimulating material" (Barenkamp, p. 7,111 to 5; Loosmore, col. 3,11 63 to 65).

The only "immunogenic or immunostimulating material" identified is an adjuvant, suggesting that the latter materials are preferred additional components, rather than an immunogenic material. In any event, there is no immunogenic material particularly specified in either reference and neither does the Examiner suggest that there is.

The two references also contain the statement:

> "A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen or from combinations of various pathogens" (p. 22, II 1 to 8 of Barenkamp; p. 9, II 14 to 19 of Loosmore).

While suggesting various combinations, there is no suggestion here to combine different proteins derived from the same pathogen, as in applicants claim 6. Again, the references are silent as to any specific combination contemplated.

The Examiner's view is best summarized by the statement in the Office Action that:

> "No more than routine skill was required at the time of appellants invention to combine two well-known compositions, i.e. two different antigens of *H. influenzae,* each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for that very same purpose of providing an immunogenic composition."

However, the cited prior art lacks the motivation to do so. As noted above, there are vague, non-specified indications in both references to combine other components with the specific immunogen, but there is no specific indication as to what that other component may comprise, other than an adjuvant (first quotation above) or materials from the pathogens and/or materials from various strains of the same pathogen (second quotation above).

As the Examiner has pointed out, on page 49, lines 15 to 19 of Barenkamp, it is stated:

> ".... the data suggests the HMW adhesin proteins are potentially important protective antigens which may comprise one component of a multi-component NTHI vaccine."

This passage appears to suggest that only *Haemophilus* proteins which are the HMW adhesin proteins are appropriate components. The non-proteolytic analog of Hin47 is not an adhesin (although initially thought to be adhesin, see col. 2, line 17 of Loosmore et al). (It is pointed out that the Examiner is incorrect in the statement that the adhesin protein "should" comprises one component of the NTHI vaccine. As can be seen from the above quotation, Barenkamp uses the word "may").

Even if the Examiner finds motivation in this passage of Barenkamp to combine the HMW protein with another *Haemophilus* antigen, whether an adhesin or not, such motivation still provides no motivation to select the non-proteolytic Hin47 analog as the other *Haemophilus* antigen.

There have been a significant number of *Haemophilus* proteins identified as vaccine candidates besides the HMW and Hin47 analog proteins. These proteins include the various outer membrane proteins A to H, lactoferrin and transferrin receptor protein and the P1, P2, P6 and D15 proteins. It is submitted that there is no motivation provided by the cited prior art why a person skilled in the art would specifically select from all the optional possibilities, the non-proteolytic Hin47 analog to specifically combine with the HMW protein.

The Examiner states in the Office Action, quoting In re Kerkhoven, that:

> "The idea of combining them flows logically from their having been individually taught in the prior art."

The "idea of combining them" does not explain why the two materials should be combined when there is selection available. If the two antigens were the only two known antigens of *Haemophilus influenzae,* then there may be some validity to the position taken by the Examiner, but this is clearly not the case here.

In any event, caution is required when considering combining different antigens into immunogenic compositions because of the danger of impairment of the immunogenicity of the individual components one by the other. As may be seen from Applicants data, this phenomenon was observed for increasing amounts of H91A Hin47 when combined with a low dose of HMW, but disappeared at higher doses of HMW (see Figure 3).

The Examiner indicates in the Office Action that:

> "Applicant neither argues, nor shows scientific data teaching unexpected results".

It is submitted that such is not the case. Applicants data clearly shows that a synergistic effect can be achieved both in response to the HMW and H91A Hin47 by combining them. Thus, there is a synergistic effect observed for increasing amounts of HMW on the primary antibody response to a low dose of H91A Hin47. The H91A H47 improved the primary response to HMW, if the HMW was not present in low doses.

It is submitted that these findings are a surprising result. In addition, it is further surprising that HMW would enhance the vigorous antibody response to H91A Hin47, since it is a weaker immunogen.

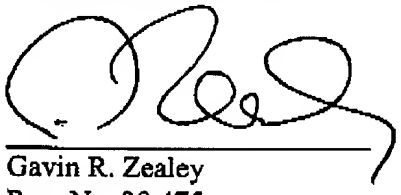
Furthermore, these results are unexpected in the field of combination vaccines. There is little expectation of success that simply mixing existing vaccine antigens will not result in incompatibilities amongst the various antigens, resulting in loss of stability or reduced potency or indeed a synergistic effect increasing potency. Immune interference cannot be predicted. Others skilled in the art of combination vaccines have found that the preparation of combination vaccines is far from straight forward. For example Cauldfield et al (2001) report on the need for a balanced formulations of vaccine components in the preparation of DTP combination vaccines to circumvent interference with the components. Van den Bosch et al (2003) have also reported that the addition of a potential antigen (Pal A) from A. pleuropneumoniae can completely eliminate the positive efficacy of known antigens (ApxI and II) when combined (see abstract).

For all these reasons, it is submitted that claims 6 to 24 are patentable over the applied art and the rejection thereof under 35 USC 103(a) as being unpatentable over Barenkamp in view of Loosmore et al.

(11) Summary

Having regard to the above detailed discussion, it is submitted that the Examiner is in error in rejecting claim 6 to 24 as being unpatentable and hence the rejection thereof under 35 USC 103(a) as being unpatentable over the combination of Barenkamp (WO 97/36914) in view of Loosmore et al, should be REVERSED.

Respectfully submitted,



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APPENDIX I

CLAIMS APPEALED (09/210,995)

6. An immunogenic composition for conferring protection in a host against disease caused by *Haemophllus influenzae,* which comprises:
an analog of *Haemophllus influenzae* Hin47 protein having a decreased protease activity which is less than about 10% of that of natural Hin47 protein, and a high molecular weight (HMW) protein of a strain of non-typeable *Haemophilus influenzae.*

7. The composition of claim 6 wherein said HMW protein is present in said composition in an amount which enhances the immune response in the host to the Hin47 protein.

8. The composition of claim 7 wherein said HMW protein is present in the said amount while the individual immunogenicities of the proteins in the composition is not impaired.

9. The composition of claim 6 wherein said analog of Hin47 protein is one in which at least one amino acid of the natural Hin47 protein contributing to protease activity has been deleted or replaced by a different amino acid and which has substantially the same immunogenic properties as natural Hin47 protein.

10. The composition of claim 9 wherein said at least one amino acid is selected from the group consisting of amino acids 91, 121 and 195 to 201 of natural Hin47 protein.

11. The composition of claim 10 wherein Serine-197 is replaced by alanine.

12. The composition of claim 10 wherein Histidine-91 is replaced by alanine, lysine or arginine.

13. The composition of claim 12 wherein Histidine-91 is replaced alanine.

14. The composition of claim 10 wherein Asp-121 is replaced by alanine.

15. The composition of claim 8 wherein said HMW protein is an HMWI or HMW2 protein of a non-typeable strain of *Haemophilus influenzae.*

16. The composition of claim 15 wherein the HMW1 and HMW2 proteins are produced recombinantly.

17. The composition of claim 15 wherein said HMW1 and HMW2 proteins are derived

from the respective strain of non-typeable *Haemophilus influenzae* and possess respective molecular weights as set forth in the following Table:

Molecular Weight (kDa)	Non-typeable *H. influenzae* Strain					
	12	JoyC	K21	LCDC2	PMH1	15
Mature Protein: HMW1	125	125.9	104.4	114.0	102.4	103.5
HMW2		120	100.9	111.7	103.9	121.9

18. The composition of claim 6 further comprising an adjuvant.

19. The composition of claim 18 wherein said adjuvant is aluminum hydroxide or aluminum phosphate.

20. The composition of claim 6 comprising about 25 to about 100 μg of the Hin47 protein analog, and about 25 to about 100 μg of the HMW protein.

21. The composition of claim 6 further comprising at least one additional antigenic component for conferring protection against infection caused by another pathogen.

22. The composition of claim 6 wherein said at least one additional antigenic component is selected from the group consisting of diphtheria toxoid, tetanus toxoid, pertussis antigens, non-virulent poliovirus and a conjugate of a tetanus or diphtheria toxoid and a capsular polysaccharide of *Haemophilus influenzae.*

23. The composition of claim 22 wherein said pertussis antigens are selected from the group consisting of pertussis toxoid, filamentous hemagglutinin, pertactin and agglutinogens.

24. A method of immunzing a host against disease caused by infection with *Haemophius influenzae*, including otitis media, which comprises administering to the host an immunoeffective amount of a composition as claimed in claim 6.

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Immunogenicity of a hexavalent combination vaccine in rhesus monkeys

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Abstract

Preclinical immunogenicity studies were conducted in rhesus monkeys to determine whether there is immune interference in the response to one or more components of a hexavalent vaccine (Hexavac™) that contains antigens from *Haemophilus influenzae* (Hib), hepatitis B (HB), diphtheria (D), tetanus (T), acellular pertussis (aP) and inactivated polio virus (IPV). Antibody responses were measured following co-administration of the components at three separate anatomical sites or administration as a hexavalent combination in a single site. After three injections of the hexavalent vaccine, the peak antibody responses to each component of the vaccine were > 100-fold above pre-immune titers and persisted at levels > 10-fold above pre-immune titers at ≈ 1 year. Immune interference was observed in the peak response to HB, D and pertussis toxin, but was not seen at later time points. The results indicate that the rhesus monkey model may be useful for pre-clinical evaluation of combination vaccines.

Keywords: Combination vaccine; Immune interference; Antigen competition; Non-human primate

1. Introduction

The rationale for the development of combination vaccines has been discussed in recent publications [1–3]. The main benefits are to enhance compliance and vaccine coverage and to reduce overall healthcare costs. An added benefit is to 'make room' in the pediatric vaccination schedule for new vaccines projected for the new millennium [4]. However, experience has shown that the preparation of combination vaccines is far from straightforward. During the development of the DTP combination vaccine, the need for 'balanced' formulations of vaccine components was recognized [5] and careful dose-ranging of the three serotypes of the oral poliovirus vaccine (OPV) was required in order to circumvent interference of the type 2 strain on the immune response to types 1 and 3 [6]. The main impediments to the development of combination vaccines are stability and immunogenicity. Simply mixing existing vaccines can result in incompatibilities among the various antigens, adjuvants, preservatives, stabilizers and excipients, resulting in a loss of stability or reduced potency [7]. A further confounding factor is that of immune interference (also known as antigen competition) which may not always be predicted using animal models. Antigenic competition was first described by Michaelis in 1904 [8], but is still poorly understood.

The objective of the present preclinical immunogenicity studies of Hexavac™ was to determine the antibody response to vaccine component antigens at various times after immunization and to compare the response to the hexavalent vaccine with that induced by administration of Hib, HB and DTaP-IPV at separate anatomic sites. The results indicate that there was a significant difference between experimental and control arms in the peak responses to HB, D and PT. These differences fade with time and there was no significant

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difference in the response to any vaccine component tested at 48–51 weeks post-dose 1. Overall, strong antibody responses were induced to each component of Hexavac™ in rhesus monkeys.

2. Materials and methods

2.1. Experimental animals

The rhesus monkeys (*Macaca mulata*) used in this study were born at the California Regional Primate Center at the University of California at Davis and all immunizations and blood collection procedures were performed at that site. Some monkeys were housed outdoors in social groups, whereas others were maintained indoors, in pairs. Those maintained indoors had a 12:12 h light:dark cycle within a temperature range of ≈17–29°C. Animals were all fed Purina Monkey Chow, 15% protein with fresh produce supplements two to three times per week. Monkeys were identified by tattoos containing unique numbers. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC).

2.2. Vaccine composition

The hexavalent vaccine consisted of Hib capsular polysaccharide (polyribosyl ribitol phosphate) conjugated to tetanus toxoid (PRP-T), hepatitis B (HB), surface antigen (HBsAg), diphtheria toxoid (D), tetanus toxoid (T), pertussis filamentous hemagglutinin (FHA), pertussis toxoid (PT) and three serotypes of inactivated poliovirus (IPV) formulated with aluminum adjuvants. Each 0.5 ml dose contained 12 μg PRP-T (expressed in polyoside), 5 μg HBsAg, 30 Lf D, 10 Lf T, 25 μg FHA, 25 μg PT, IPV type 1 (40 D-Ag U), type 2 (8 D-Ag U) and type 3 (32 D-Ag U).

2.3. Vaccination/schedule

One group of rhesus monkeys was immunized with half of the human pediatric dose of the hexavalent combination vaccine (Hexavac™) into a single i.m. site, while a second cohort (control group) of monkeys was injected with half of the human dose of PRP-T (ActHIB®), HBsAg (RECOMBIVAX HB®) and DTacP-IPV at three separate i.m. sites at 0, 4 and 8 weeks, according to the protocol shown in Table 1. The monkeys were weighed at each time point and examined for injection site reactions after each dose of vaccine. In addition, blood samples collected at each time point were monitored for changes in white cell, red cell and platelet levels.

2.4. Serological assays

Sera were collected at week 0, 4, 8, 10 and 48–51 and tested individually for antibody titers against PRP, HBsAg, D, T, FHA and PT. Due to a shortage of sera, serology was not performed to detect antibodies against poliovirus. Anti-PRP (component of Hib) antibody titers were measured using a Farr-type radioimmunoassay (RIA), as previously described [9,10]; responses to HB were determined using a modified Ausab® assay (Abbott Laboratories, N. Chicago, IL), as described elsewhere [11]. Antibodies against FHA [12] and antibody titers against T were measured by ELISA [13]. Diphtheria toxoid antibody titers were assayed by using a neutralization test in comparison to a WHO antitoxin standard [14]. PT antibody titers were also determined by a toxin neutralization test on CHO cell culture [15]. The results are expressed as geometric means. The assays used were originally validated for analysis of human samples and adapted for testing monkey serum without further analytical validation.

2.5. Statistical analysis

At each time point, the estimated GMT ratio for the experimental vaccine group relative to the control group (Hexavac™/Hib + HB + DTaP-IPV) and corresponding two-sample 99% confidence interval for the true GMT ratio are calculated for the response to each antigen, assuming unknown but equal variances between the two groups. If a particular interval excludes

Table 1
Protocol for preclinical immunogenicity testing of Hexavac™ in rhesus monkeys

Group	n	Age at dose 1 (months)	Vaccine	Injection schedule (weeks)	Bleeding schedule (weeks)
1	8[c]	6–12	Hexavac™[a]	0, 4, 8	0, 4, 8, 10, ≈50
2	8	4.8–10.5	ActHIB[a] + RECOMBIVAX HB[a] + DTaP-IPV[b]	0, 4, 8	0, 4, 8, 10, ≈50

[a] Injection volume of 0.25 ml in one intramuscular site.
[b] Injection in three separate intramuscular sites (0.25 ml each).
[c] Serum samples from six of eight monkeys were available at the week ≈50 time point.

the value 1, the corresponding comparison between Hexavac™ and Hib + HB + DTaP-IPV is statistically significant; otherwise, it is not. The reason for using a 99% confidence level instead of the usual 95% level is to control the overall false-positive rate (per antigen), which is defined as the probability that at least one of the confidence intervals will exclude the value 1 by chance alone [16].

3. Results

3.1. Serum antibody response to vaccination

Antibody titers to each component of the vaccine (except IPV) were measured at week 0, 4, 8, 10 and 48–50 using sera from individual animals (Fig. 1). At week 4 (post-dose 1), there were no significant differences between groups in antibody titers to any of the antigens tested. Similarly, at week 8 (4 weeks post-dose 2), there were no significant differences among groups with the exception of the response to HB, which was significantly higher in the control group compared with the monkeys injected with the hexavalent combination. As shown in Table 2, at the 10-week time point (2 weeks post-dose 3), there was a significantly higher response to three of the vaccine components (HB, D and pertussis toxin) in monkeys immunized with separate injections of Hib + HB + DTaP-IPV compared with the response of monkeys immunized with the hexavalent combination vaccine. Importantly, at the final time point (week 48–51), which is 38–41 weeks post-dose 3, there was no significant difference in the response to any component of the vaccines.

3.2. Response rate to vaccination

The percentage of responders to components of the vaccine was determined at each bleed time point. In the absence of established 'seroprotective titers' for rhesus monkeys, the accepted human equivalents were used as shown in the legend to Table 3. For pertussis, there is no proven correlate of protection established for humans, therefore, the percentage of seroconverters was used instead. As shown in Table 3, there was no difference in the response rate to vaccination with Hexavac™ compared with separate site administration of Hib + HB + DTaP-IPV except for the response to HB at the 8-week time point. At that time, only 37% (3/8) monkeys responded to Hexavac™ whereas 100% (8/8) responded to the control. These results are consistent with the analysis of the serological titers that showed a significant difference in anti-HBs titers at this time.

3.3. Adverse event monitoring

Animals were monitored for changes in weight or blood cell counts as well as for injection site reactions. No adverse reactions were noted at the site of injection at any time point and there was no adverse effect of vaccination on the weight or blood cell counts of any animals (data not shown).

4. Discussion/conclusion

The results from the present pre-clinical evaluation of Hexavac™ indicate that there was a vigorous response to each component of the vaccine. Even so, there was evidence for interference in the peak response to HB, D and pertussis toxoid when the responses to Hexavac™ were compared with the control group. Four types of immune interference (antigen competition) have been described: (a) sequential; (b) intramolecular; (c) intravirionic; and (d) intermolecular competition.

- Sequential competition occurs when a second antigen (or vaccine) is given shortly after a first antigen (or vaccine) [17]. This form of interference is especially relevant to vaccine dosing schedules.
- Intramolecular competition results from competition among peptides derived from the same protein for binding to MHC Class I or Class II molecules [18].
- Intravirionic competition results when one protein antigen within a virus interferes with the response to a second protein antigen within the same virus [19]. This form of antigen competition can be circumvented by dissociation of the virus into its component parts prior to immunization.
- Intermolecular competition results when one antigen in a mixture interferes with the immune response to a second antigen in a mixture [17,20]. This form of interference is most relevant to the present investigation; however, the mechanism by which this happens is unknown. One possibility is that one or more components within the combination vaccine become unstable, perhaps due to excipients carried over into the vaccine with a separate component. However, extensive stability studies have been performed on Hexavac™ and the components that had reduced immunogenicity in the combination vaccine (HB, D and PT) were shown to be stable for several years (data not shown). Thus, the decreased response to certain of the vaccine components of Hexavac™ does not appear to be related to a loss of stability of these components, suggesting that the explanation is immune interference due to intermolecular antigen competition.

Although the rhesus monkey model suggests that the response to Hexavac™ is marked by transient interference in response to the HB, D and PT components,

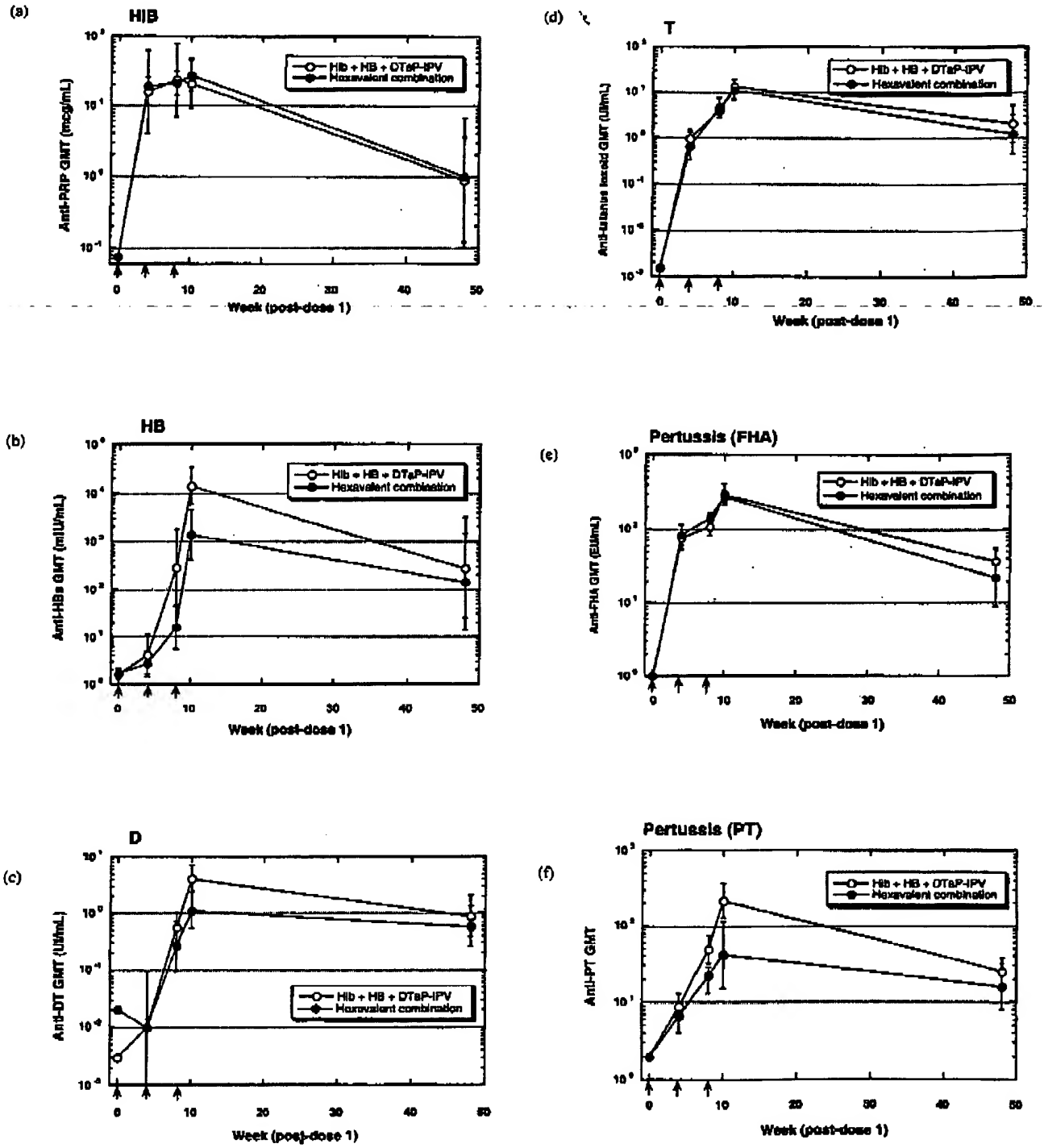

Fig. 1. Antibody response to antigens from Hib, HB, D, T, FHA and PT in rhesus monkeys immunized with Hib + HB + DTaP-IPV at separate sites or with the hexavalent combination vaccine (Hexavac™) at a single i.m. site. Monkeys were immunized at week 0, 4 and 8 and antibody titers were determined on serum collected at week 0, 4, 8 and ≈ 50. Results are expressed as the geometric mean with 95% confidence intervals.

these apparent differences in potency are unlikely to translate into clinically meaningful differences since: (a) the response to each component of the vaccine is > 100-fold higher than the pre-immune titers; (b) the response rate post-dose 3 (percent seroconverters) is equivalent in the two groups; and (c) the difference in titers elicited by Hexavac™ and the control arm became indistinguishable over time. By study week 48–51,